

# Enzymatic breakage of dimethylsulfoniopropionate - a signature molecule for life at sea

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## Abstract

Largely using gene-based evidence, the last few years have seen real insights on the diverse ways in which different microbes break down dimethylsulfoniopropionate, an abundant anti-stress molecule that is made by marine algae, some corals and a few angiosperms. Here, we review more recent advances in which *in vitro* biochemical tools – including structural determinations – have shed new light on how the corresponding enzymes act on DMSP. These have revealed how enzymes in very different polypeptide families can act on this substrate, often by novel ways, and with broader implications that extend from enzymatic mechanisms to microbial ecology.

## Introduction

The sulfur-containing zwitterion dimethylsulfoniopropionate (DMSP), is made in massive amounts ( $\sim 10^9$  tons annually, worldwide), with influences on diverse phenomena, from cloud formation to bird behavior [1]. Yet there is less general awareness of DMSP than these credentials deserve, most likely because it is a molecule of the seas and their margins. The marine organisms that make DMSP embrace single-celled plankton as diverse as dinoflagellates, diatoms and coccolithophores, as well as some red and green algal seaweeds, a few angiosperms [2], and, recently, the first DMSP-synthesizing animal, the coral *Acropora* was described [3]. DMSP may serve as an osmoprotectant, though other, anti-stress functions have been suggested (e.g. [4]); indeed, it may have different duties in the varied organisms that make it.

Although first identified in 1948 [5], we know little of how DMSP is made at a molecular genetic level, though some credible pathways were proposed (e.g. [6]). In contrast, there have been recent insights into how DMSP is catabolized, although awareness of this phenomenon, too, is not new. Cantoni and Anderson [7] showed in 1956 that the DMSP-producing macroalga *Polysiphonia* also broke it down to acrylate and the volatile dimethyl sulfide (DMS), as do other eukaryotic DMSP-producing organisms (see [8]). Furthermore, many marine bacteria can concentrate the DMSP that leaks from producer organisms, and then catabolise it. This process is not only a major driver of marine nutrition and global sulfur cycling, but the DMS product itself has diverse environmental effects. It is a chemoattractant

for marine animals (birds, mammals and invertebrates), as indeed is DMSP itself [9,10]. And, at a very different level, DMS oxidation products cause atmospheric water molecules to coalesce, with possible effects on weather through cloud formation [11].

Furthermore, some abundant marine bacteria can catabolize DMSP in a very different way, in which the substrate is first demethylated in a pathway that allows some bacteria to use DMSP as a sulfur and/or carbon source [12-15].

This chapter encapsulates recent work on DMSP catabolism, emphasizing biochemical studies on the relevant enzymes, relating these to earlier genetic analyses (recently reviewed [12-15]).

### **DMSP catabolism by eukaryotic phytoplankton**

*Emiliania huxleyi* is an abundant single-celled coccolithophore, long known not only to make DMSP, but to cleave it into DMS plus acrylate, possibly as a defense mechanism against zooplankton predation [16]. But, only very recently has the corresponding gene and its DMSP lyase product been identified [8]. This nuclear gene, *Alma1*, encodes a tetrameric DMSP lyase that is sensitive to oxidative stress and is located in the cell's chloroplastic membrane [8]. *Alma1* is in the aspartate/glutamate racemase super-family, which not only includes racemases *sensu strictu*, but has representatives with different functions, for example aryl-malonate decarboxylases and maleate *cis-trans* isomerases. These enzymes add or remove a proton from the carbon adjacent to a carboxylate, so *Alma1* likely catalyzes the abstraction of a proton next to the DMSP carboxylate, releasing DMS plus acrylate (Figure 1).

An active DMSP lyase with low-level similarity (~25% identical to *Alma1*) also occurs in the dinoflagellate *Symbiodinium*, a distantly related phytoplankton that interacts intimately with its Cnidarian host and is essential for coral productivity [17]. The functions of a range of polypeptides with similarly low sequence identity to *Alma1* but which occur in diverse phytoplankton and even in bacteria are yet to be established. Interestingly, *E. huxleyi* itself has other poorly conserved *Alma1* paralogues; those that were tested had very low DMSP lyase activity but the possibility that these were not expressed in soluble, fully functional form was not excluded [8]. One of these comprises two, tandemly arranged *Alma1*-type domains, superficially reminiscent of the architecture of the bacterial DMSP lyase Dddd (below).

*E. huxleyi* strains differ in their baseline levels of DMS production and, nicely, this correlates with their amounts of *Alma1* protein [8]. However, the intrinsic and/or extrinsic signals that affect expression of *Alma1* are unknown. We did note, however, that published transcriptomic measurements indicated that *E. huxleyi Alma1* and the *Symbiodinium Alma1*-like gene were expressed at low levels in laboratory-grown cells. Expression was unaffected by temperature or salinity [18,19], although transcription of *E. huxleyi Alma1* was enhanced ~3-fold in low-sulfate media [18,19].

### **Bacterial DMSP catabolism**

It was known for some time that DMSP could be catabolized by bacteria in two distinct ways – the “demethylation pathway” in which a methyl group is removed, yielding methylmercaptopropionate (MMPA), and a “lysis” route in which DMS was produced, with some marine bacteria being able to accomplish both of these (see [16]).

To date, only one gene (*dmdA*) that encodes a DMSP demethylase enzyme has been identified; in contrast, the lyases, encoded by different *ddd* genes are of at least four different types (see [12,13,20]). We now consider recent biochemical analyses of these DmdA and Ddd gene products, summarized in Table 1.

### **DmdA DMSP Demethylase**

The DMSP demethylase, DmdA, is found, mostly, in two clades of marine alpha-Proteobacteria, namely the Roseobacters and *Pelagibacter ubique* (SAR11), [21,22], both of which are very populous in the oceans, accounting for the widespread and abundant distribution of *dmdA* genes in marine metagenomes. Our own inspection of the large (>4x10<sup>7</sup> non-redundant sequences) Tara Oceans data set [23] showed that ~28% of the sampled cells contained *dmdA*, a similar value to that found in surveys of other metagenomes [22], notably that of the Global Ocean Survey (GOS) [24].

DmdA has significant (25%) sequence identity to amino-methyltransferases of the glycine cleavage T-protein (GVT) family. In addition to their deamination activity, these enzymes transfer a methyl group to the acceptor molecule, usually tetrahydrofolate (THF) to yield 5, 10-methylene-THF. Atypically for members of this family, though, the methylated product of the DmdA-mediated demethylation of DMSP is 5-methyl-THF [25].

The recently obtained structure [26] of *P. ubique* DmdA shows that it is a dimer and although it adopts a fold that resembles other GVT family members, there are significant differences at both the THF- and nearby DMSP-binding sites. The structure revealed the proximity (3.3 Å) of the N5 atom of THF and the methyl group on the DMSP’s sulfonium atom, prompting a model that couples the methyl transfer (to THF) and the N5 proton to form a water molecule. Mechanistically, this more closely resembles that in the family of SAM-dependent methyltransferases, rather than that of other GVT enzymes. Thus, minor, precise differences near the active site of GVT family enzymes can affect the specificity and the enzymatic mechanism, even though the overall fold is little-changed.

### **DMSP Lysis Pathway(s)**

The lysis pathway can be mediated by at least four classes of “Ddd” (DMSP-dependent DMS) bacterial enzymes, representing different polypeptide families.

- DddD Acetyl CoA Transferase

The *in vitro* characterization of the DddD DMSP lyase [27] substantiates and extends earlier genetic and bioinformatic observations on this enzyme, identified first in the  $\gamma$ -Proteobacterium *Marinomonas* [28]. DddD is (so far) unique in that its C3 initial product is not acrylate, but 3-hydroxy-propionate (3HP), or a CoA-linked version thereof [28] (Figure 1).

The DddD polypeptide has an unusual architecture, with similar, tandemly arranged domains. These both resemble CaiB, a Class III CoA-transferase involved in interconverting crotonobetaine and *L*-carnitine, the latter being structurally similar to DMSP [29]. DddD's catalytic mechanism likely involves an initial, acetyl-CoA-dependent covalent attachment of DMSP to the enzyme, possibly at an aspartate residue that is conserved at the C-terminal domain of all functional DddD enzymes [27]. Subsequent hydration of DMSP releases DMS plus 3HP-CoA (and/or free, unmodified 3HP). While resembling CaiB, both in sequence and in some of the proposed mechanisms, DddD has distinctive features. CaiB forms a homodimer of intertwining polypeptides [30]; although DddD may have a similar overall structure, this would comprise a single, larger polypeptide [27]. Also, CaiB CoA-transferase works in conjunction with a second enzyme, CaiD, which is responsible for hydrating carnitine CoA; in contrast, and unusually, DddD accomplishes both these reactions. Many bacteria containing DddD (mainly  $\gamma$ -Proteobacteria, e.g. *Marinomonas*) grow well on DMSP as sole carbon source [28,31]. Furthermore, *dddD* is often clustered with *dddA* and *dddC*, which encode enzymes needed for subsequent, sequential catabolism of 3HP(-CoA) [28,31], consistent with DddD's important role in DMSP-dependent nutrition. However, functionally ratified homologues of DddD also occur sporadically in other sub-phyla of Proteobacteria – e.g. Rhizobiales and Rhodobacterales (both  $\alpha$ -) and Burkholderiales ( $\beta$ -) - pointing to repeated horizontal gene transfer (HGT) events [28]. This DddD-type architecture of two tandemly arranged CaiB domains is widespread in different bacterial taxa, albeit their sequences being less similar to those in DddD itself. The function(s) of these homologues is unknown. Further, it was noted [32] that *E. huxleyi* has a gene that would encode such a polypeptide. However, this does not appear to be a *bona fide* DMSP lyase, not least because this eukaryote makes acrylate, not 3HP, as its C3 DMSP cleavage product (see above).

- The “metallo-peptidase” DddP

The DddP DMSP lyase cleaves DMSP into DMS plus acrylate [33,34], and is widespread in bacteria of the Roseobacter and SAR116 clades, accounting for its relatively high abundance (4-26% of sampled bacterial cells) in a number of marine metagenomes [22,35-37]. Functional DddP enzymes also exist in a few strains of other bacteria and, strikingly, in much more distantly related organisms, even including Ascomycete fungi, likely through inter-Domain HGT [33,38]. As predicted from its sequence [33] the recently described structures of DddP in two Roseobacters confirm it to be in the M24B sub-group of metalloproteases [39,40], with the expected metalloproteinase-like ‘pitta-bread’ fold. It is dimeric [34], with the interface of the two monomers encompassing the active sites – one per monomer. Each site includes a binuclear metal centre, one occupied by Fe and the other by a choice of different metals. The two metal ions are co-ordinated to residues (3 x aspartate, 2 x glutamate and a histidine) that are conserved in all functional DddP enzymes (see Figure 4 in [39,40]). Other conserved residues (tryptophan and tyrosine) of DddP are proposed to bind to the S<sup>+</sup> of DMSP via cation- $\pi$  interactions and orientate this substrate for electrostatic

interaction with one Fe ligand at the carboxyl group, causing the Fe to move [40]. This ion-shift would expose the remainder of the substrate to two residues (aspartate and glutamate) that would abstract a proton, leading to a beta-elimination, releasing DMS and acrylate. The model tallies with earlier site-directed substitutions of the predicted metal-binding amino acids; these completely abolished DMSP lyase activity [34].

- Cupins

The cupin motif comprises a metal-binding  $\beta$ -barrel and occurs in a vast number of polypeptides, with myriad functions [41]. No fewer than three different cupin-type DMSP lyases, DddL, DddQ and DddW have been identified to date – again, mostly in the marine Roseobacters [12]. These lyase enzymes share no extended amino acid similarity, except at this cupin motif, positioned towards their carboxyl termini.

The recently presented structure [42] of DddQ and biochemical work on DddW [43], two cupin-type DMSP lyases respectively from *Ruegeria lacuscaerulensis* and *R. pomeroyi* reveal that both are dimeric. Furthermore, both depend on a divalent metal cofactor, binding to three histidines and a glutamate residue within their cupin domains. These residues are conserved in the cupin DMSP lyases (Figure 2a), and modelling, based on the DddQ structure, shows that their predicted locations in DddL and DddW are very similar to those in DddQ. The crystallised DddQ protein contains a bound Zn whereas DddW is purified predominantly containing Fe. However, both these lyases can function with other divalent metal cofactors; viz  $Mn^{2+}$  and  $Co^{2+}$  for DddQ and  $Mn^{2+}$  and  $Fe^{2+}$  for DddW. Despite this apparent promiscuity,  $Fe^{2+}$  is the “preferred” ion for DddW, with a  $K_d$  of  $\sim 5$  nM,  $\sim 200$ -fold lower than other tested transition metals [43].

The proposed mechanism of cupin-containing DMSP lyases [42] for beta-elimination of a proton has novel features, in that initial binding of DMSP to the metal cofactor may cause movement of a nearby, conserved tyrosine (Y131 for DddQ as shown in Figure 2b), bringing the oxygen atom of tyrosine closer to the alpha-carbon of DMSP, leading to the abstraction of a proton plus the release DMS and acrylate. It should be noted, though, that this model has been questioned by others [44].

### Closing discussion points

The last few years have seen increasing amounts of biochemical flesh on earlier genetic and physiological bones of DMSP catabolism. And, again, the watchwords seem to be “novelty” and “variety”. Even for those enzymes whose deduced amino acid sequences place them in known, and sometimes well-characterised, polypeptide families, several of them have distinctive features.

Thus:

- DddD is the first characterised enzyme with the tandem CaiB-CaiB CoA-transferase domain arrangement, maybe providing a template for other polypeptides with this architecture, in diverse organisms, but with, as yet, unknown function(s).

- The proposed role of the mobile tyrosine residue in DddW, and by inference in other cupin-type lyases, is highly unusual - though see comments by others [44].
- Although DddP is in a family whose usual substrates are amino acids, and has an overall structure that resembles archetypal M24 proteases, DMSP is clearly not a peptide. However, subtle differences in the active site residues, and the ways that these may influence the interactions with metal cofactors may account for this difference in the natures of the substrates [40].
- Similarly, the DmdA DMSP demethylase, whose overall sequence and structure place it in one family (glycine cleavage T1), has a mode of action that more closely resembles that of the SAM-dependent methyltransferases.

And, yet another “Ddd” lyase, DddY, occurs in bacteria that favour microaerobic habitats and is (so far) unique in being periplasmic [45]. It does not resemble *any* polypeptide of known function, so elucidating its enzymatic details presents extra challenges, even though it was the first to have been purified [46] and even partially sequenced [47].

It will be of interest to establish if the varied types of enzymes that act on DMSP (and/or the organisms that harbour them) are adapted to function in particular marine environments. For example the local, perhaps transient, availability of particular metals may influence the effectiveness of the different metal-containing lyases.

Many enzymes that act on DMSP have strikingly (some might say “disturbingly”) high  $K_m$  values, in the millimolar values for DddP and DmdA and especially high for the cupins (Table 1). Also troubling are the low  $K_{cat}$  values of some of the cupin lyases. Some authors (including ourselves [12]) asked if this hints that the “real” substrate for at least some of them may not be DMSP, but other, as yet unknown molecules [44]. Even though bacteria can concentrate DMSP to impressively high intracellular concentrations – as much as 70 mM [25] – the concerns raised by the values of these catalytic parameters still require unambiguous resolution. Of course, the question of what is the “correct”, natural substrate for any given enzyme is not restricted to those that include DMSP as a substrate, and the consequences and origins catalytically promiscuous enzymes have been considered in some depth (e.g. [48]). Although some of these “DMSP lyases” may indeed turn out to be other than this term implies, the fact that their corresponding genes are seen in the metagenomes of bacteria that are from the seas and their margins and not, for example, in soils or in the human microbiome, strongly predicts that the “correct” substrate (if such there be) also has a marine distribution. For the moment at least, we go along with the inestimable Douglas Adams, who shrewdly noted that *“If it looks like a duck and quacks like a duck, we have at least to consider the possibility that we have a small aquatic bird of the family Anatidae on our hands”* [49].

In any event, although our understanding of how DMSP is catabolised has progressed significantly, on a number of fronts, there are still questions to be answered, ranging from enzyme mechanisms to microbial ecology.

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## Figure Legends

### Figure 1. Products formed through the action of different enzymes that act on the substrate DMSP.

Dimethylsulfoniopropionate (DMSP) is converted to acrylate, dimethyl sulfide (DMS) and a proton via the DddL, Q, W, Y, P and Alma1 enzymes of the DMSP lyase pathway. The DddD enzyme of the CoA DMSP lysis pathway produces 3-hydroxypropionate (3HP)-CoA and DMS from DMSP, using acetyl-CoA as a CoA donor. The demethylation of DMSP by DmdA produces methylmercaptopropionate (3-MMPA) and methyl-tetrahydrofolate (THF). Bacterial enzymes are in pink background, eukaryotic in green, and DddP, found in both Domains, in yellow. The protein families of each enzyme are indicated.

### Figure 2. Cupin motifs and metal binding residues of DddL, DddQ and DddW.

(A) Sequence alignment of cupin regions of selected DddL, DddQ and DddW proteins using sequences at NCBI and Geneious [50]. The two conserved cupin motifs 1 (GX<sub>5</sub>HXHX<sub>3,4</sub>EX<sub>6</sub>G) and 2 (GX<sub>5</sub>PXGX<sub>2</sub>HX<sub>3</sub>N) are boxed in red. The catalytically important, metal-binding histidine and glutamic acid residues are highlighted in green. The conserved Tyr residue shown to play a catalytic role in DddQ is marked in cyan. Other non-variant residues are colored yellow. The sequences are from: Q1 = DddQ, *Ruegeria lacuscaerulensis* (SL1157\_0332); Q2= DddQ, *Ruegeria pomeroyi* DSS-3 (SPO1596); W1= DddW, *Ruegeria pomeroyi* DSS-3 (SPO0453); L1= DddL, *Sulfitobacter* sp. EE-36 (EE36\_11918); L2= DddL, *Rhodobacter sphaeroides* 2.4.1 (RSP\_1433). The numbers refer to the amino acid residues in *R. lacuscaerulensis* DddQ.

(B) The Zn(II)-bound structure (PDB 4LA2) [42] of *Ruegeria lacuscaerulensis* DddQ (magenta) was the template to model the active sites of DddL of *Sulfitobacter* sp. EE-36 (green) and DddW of *Ruegeria pomeroyi* DSS-3 (cyan), using SWISS-MODEL [51]). The catalytic residues H123, H125, E129, Y131 and H163 of *R. lacuscaerulensis* DddQ superimpose well in all three polypeptides. The figure was drawn using Pymol (PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC).

**Table 1. Properties of DMSP lyases and DMSP demethylase DmdA.**

Enzyme	DMSP Breakdown Products	Polypeptide class	Structure known?	Size of polypeptide and quaternary form if known	Co-factors	Enzyme $K_m$ for DMSP	Taxonomic distribution
DmdA demethylase	methylmercaptopropionate (MMPA) + methyl group, transferred to tetrahydrofolate (THF), yielding 5-methyl THF	Glycine cleavage T-protein	Yes, from <i>Pelagibacter ubique</i> [26]	~360 amino acids Mr ~ 40kDa dimer	THF used as methyl acceptor	<i>Ruegeria pomeroyi</i> 5.4 +/- 2.3 mM [25]; <i>Pelagibacter ubique</i> 13.2 +/- 2.0 mM [25]	Mainly in Roseobacters and <i>Pelagibacter</i> [SAR11] (both marine $\alpha$ -Proteobacteria); also in some marine $\gamma$ -Proteobacteria
ALMA1 "lyase"	acrylate + dimethyl sulfide (DMS) + proton	Aspartate/glutamate racemase super-family	No	<i>Emiliana huxleyi</i> ~350 amino acids MW ~ 38 kDa tetramer <i>Symbiodinium</i> ~280 amino acids Mr ~ 31 kDa	None known	<i>Emiliana huxleyi</i> 9.0 +/- 0.9 mM [8]	Eukaryotic Cocolithophore <i>Emiliana huxleyi</i> and Dinoflagellate <i>Symbiodinium</i>
DddD "lyase"	3HP-CoA + Acetate* + DMS (*; derived from acetyl CoA)	Class III acetyl CoA-transferase	No, but see [27] for model in <i>Marinomonas</i> MWYL1 DddD	~840 amino acids; MW ~94 kDa monomer of two tandemly linked CaiB-like domains	None known	Not reported for DMSP but 67 +/- 12 $\mu$ M for acetyl CoA [28]	$\gamma$ -Proteobacteria, especially in Oceanospirillales and Pseudomonadales. Also in some other Proteobacteria – Rhizobiales and Rhodobacterales (both $\alpha$ ) and Burkholderiales ( $\beta$ )
DddY "lyase"	acrylate + DMS + proton	None known	No	~400 amino acids in mature form after removal of ~21	None known	<i>Alcaligenes faecalis</i> 1.4 mM [47];	Periplasmic protein found sporadically in Proteobacteria that favor microaerobic

				amino acid N-terminal leader; MW ~45 kDa		<i>Desulfovibrio acrylicus</i> 0.4 mM [52]	environments, including <i>Alcaligenes</i> , <i>Arcobacter</i> (both $\beta$ ), <i>Desulfovibrio</i> ( $\delta$ ), <i>Shewanella</i> ( $\gamma$ )
DddP "lyase"	acrylate + DMS + proton	M24 metalloproteinase	Yes, from <i>Roseobacter denitrificans</i> [39,40] and <i>Ruegeria lacuscaerulensis</i> [39,40]	~390 amino acids; MW ~44.5 kDa dimer	Divalent metals; mostly Fe, but also Ni (see both Figure 2 and Table S1 in [39,40] )	<i>Roseovarius nubinhibens</i> 13.8 +/- 5.5 mM [34]	Mostly in "Roseobacters" Sporadically in $\gamma$ -Proteobacteria (e.g. <i>Vibrio</i> , <i>Pseudomonas</i> , <i>Oceanimonas</i> . Notably, functional DddP enzyme found in Ascomycete fungi – including <i>Aspergillus</i> and <i>Fusarium</i>
DddL "lyase"	acrylate + DMS + proton	Cupin	No	~ 230 amino acids; MW ~26 kDa	Not Tested	Not tested	Rhodobacterales – mainly in Roseobacters
DddQ "lyase"	acrylate + DMS + proton	Cupin	Yes, from <i>Ruegeria lacuscaerulensis</i> [42]	~200 amino acids; MW ~ 22 kDa dimer	Zn	<i>Ruegeria lacuscaerulensis</i> 21.5 +/- 6.8 mM [42]	Roseobacters
DddW "lyase"	acrylate + DMS + proton	Cupin	No, but see [43] for <i>in vitro</i> analyses for <i>Ruegeria pomeroyi</i>	~150 amino acids; MW ~ 16 kDa	Fe(II)	<i>Ruegeria pomeroyi</i> 8.7 +/- 0.7 mM [43]	A few Roseobacters and other Rhodobacterales e.g. <i>Ponticoccus</i>

Figure 1.

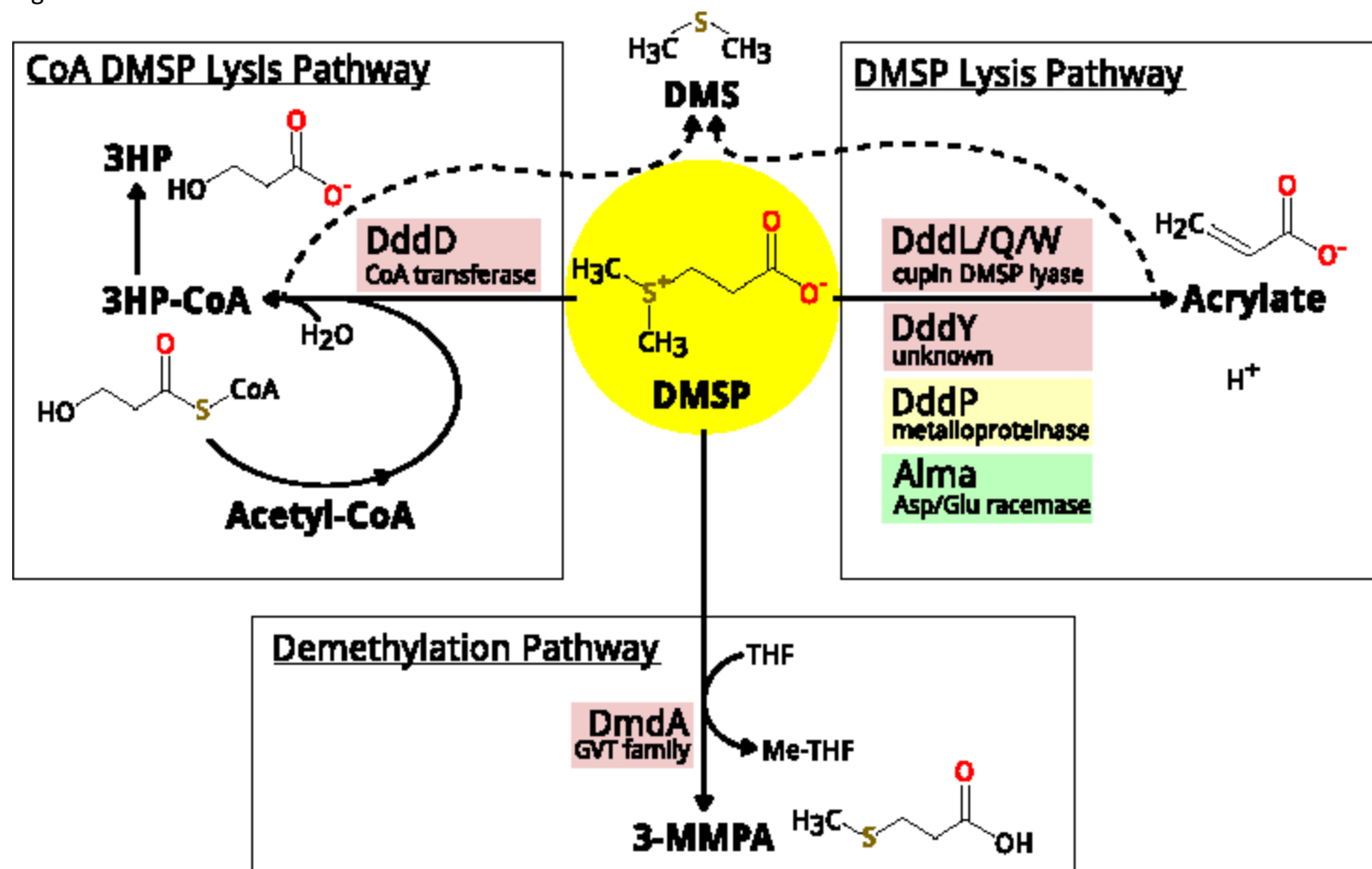


Figure 2a.





Figure 2b.

